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14. ABSTRACT We have designed and fabricated flexible electrode array system and demonstrated that the epidural stimulating arrays work better and can facilitate locomotion significantly earlier after spinal cord injury than conventional wire epidural stimulating electrodes. We have been able to maintain a functional, implanted array in a complete spinal rat for 6 weeks. We found stable parameters of the electrode array (impedance of electrodes and thresholds of evoked responses) for this period and observed stable rhythmic hindlimb activity facilitated with stimulation from selected electrode combinations. We also found that stimulation with the epidural electrode array could selectively activate spinal pathways responsible for monosynaptic and polysynaptic responses that may provide new perspectives in the assessment of the spinal cord after injury.					
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The following Specific Aims were stated in the original proposal for the reported period:

Aim 1: Develop a chronically implantable system for high density ES of the lumbosacral spinal cord.

Aim 2: Characterize the basic properties of the stimulating array developed in Aim 1, and establish effective stimulation protocols for enabling stepping in SCI rats using these high-density EAs.

Aim 3: Test the hypothesis that high density ES, coupled with robotically-guided physical training will result in better stepping performance than ES alone or robotic training alone.

B. Studies and Results

Overall summary of results

The main objective of the present work is the development of new technologies and new techniques aimed at facilitating standing and stepping recovery in rat models (and eventually humans) after spinal cord injury using a novel high density epidural stimulating array approach. Our practical goal during is to develop and demonstrate a novel high density epidural spinal stimulating array technology, demonstrate that it can be chronically implanted in a rat model, and show that high density epidural stimulation (in combination with physical therapy and pharmacological intervention) can facilitate locomotion recovery in the rat model after a complete mid-thoracic spinal cord transection. We have been able to successfully complete most of the proposed goals.

Aim 1:

- We have successfully designed and fabricated the 3x9 flexible ES array system.
- We have tested the mechanical and electrical properties of the arrays on the laboratory bench-top.
- We successfully interfaced the array with multichannel connectors, an extension cable, and a multichannel head connector system developed for the rat model.
- We found that with developed design we able to maintain implanted arrays in complete spinal rats at a fully functional level for 6-8 weeks. Stable parameters of electrode array (impedance of electrodes and thresholds of evoked responses) were observed during tested period.

Aim 2:

- We have tested the arrays *in vivo* and found that the electrode array system can be used to induce effective bilateral stepping of the hindlimbs after a complete mid-thoracic spinal cord transection.
- We found that the epidural stimulating arrays work better and can facilitate locomotion significantly earlier after spinal cord injury than conventional epidural stimulating electrodes and stimulation with array produce stable rhythmic hindlimb activity facilitated from all tested animals.

Aim 3:

- We have developed a spinal cord stimulation system integrated with multielectrode array design.
- We have identified new algorithm for spinal cord stimulation with array and machine learning paradigms that have the potential to more rapidly find the optimal array stimulation parameters.

Aim 1: Develop a chronically implantable system for high density ES of the lumbosacral spinal cord.

1. System for high density ES design.

Our current design of the implant is shown in **Figure 1**. The design involves a microfabricated electrode array and hand-assembled components to address the various technical issues of a chronic implant in a live rat. The electrode array is fabricated with a sandwich structure of parylene-metal-parylene. Parylene is a USP class VI biocompatible material, and its mechanical properties allow the necessary flexibility to make good epidural contact with the spinal cord. The metal layer is PVD-deposited platinum with a titanium adhesion layer, and 27 electrodes are arranged in 9 rows that are 3.3 mm apart with 3 electrodes in each row placed 1.0 mm apart. Other components in the assembly include a spinal baseplate to interface with the array, a custom headplug with high-density connectors, and a gold wire bundle to connect the headplug and baseplate. Medical grade epoxy and silicone are used to seal and strengthen the implant.

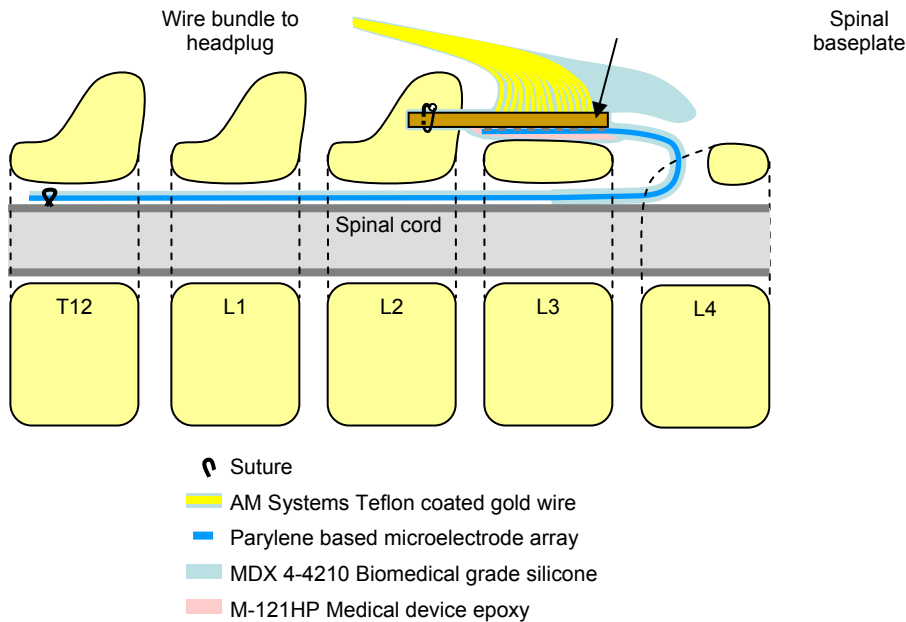


Figure 1. Current design of the chronic array implant.

This design summarizes all of the improvements we have made based on our previous failures.

In the first design of the spinal cord implant we attempted to use one thin film microfabricated device to cover the entire distance between the headplug and the spinal cord. In a chronic experiment, however, the movement of the rat placed excessive stress on the thin film device and damaged the conductive traces to the headplug. While protection of the device in silicon was considered, it was determined that it would be insufficient and the implant should be constructed with minimal stress on any microfabricated, thin-film part of the implant. Examination of the device explanted from the initial experiments showed that the largest stress was placed on the section outside the spinal cord. As a result, it was determined that a bundle of wires should form connections between the headplug and the spinal baseplate. This baseplate would be used to interface between the wires and the microelectrode array, and fixing it to the spine would minimize the mechanical stress placed on the microelectrodes. Two implant configurations were considered as shown in **Figure 2**, and both used a baseplate with a fork that could be fixed to a spinous process. It was determined that the use of a U-turn places less stress on the array because motion of the spine is not amplified through a lever action. These considerations determined the basic implant layout illustrated in **Figure 1**.

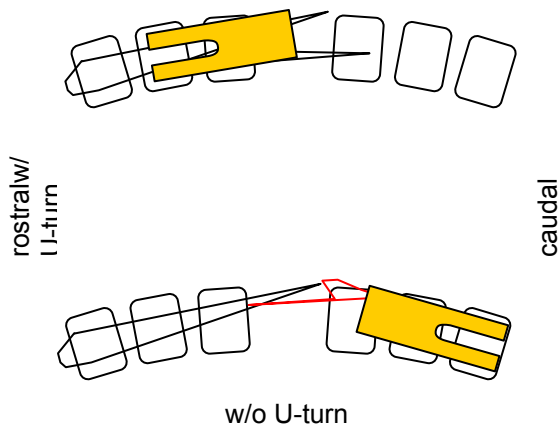


Figure 2. Two possible baseplate orientations.

Figure 3 shows two other design revisions. While there was moderate success with the design on the left, implant reliability was found to be a problem. Some devices showed partial or complete tears at the point marked B, although it was difficult to determine if the cause was the explantation procedure itself. Explants also showed considerable stress concentration at the points marked A and B, damaging the conductive traces

inside the array. The design on the right of **Figure 3** shows modifications that were made to address these issues. A thicker silicone layer was preformed into the final shape and used around the U-turn (marked as C). Although this protective silicone layer could not be applied to the ventral side of the array without interfering with the electrode interface, it could be extended along the entire dorsal side.

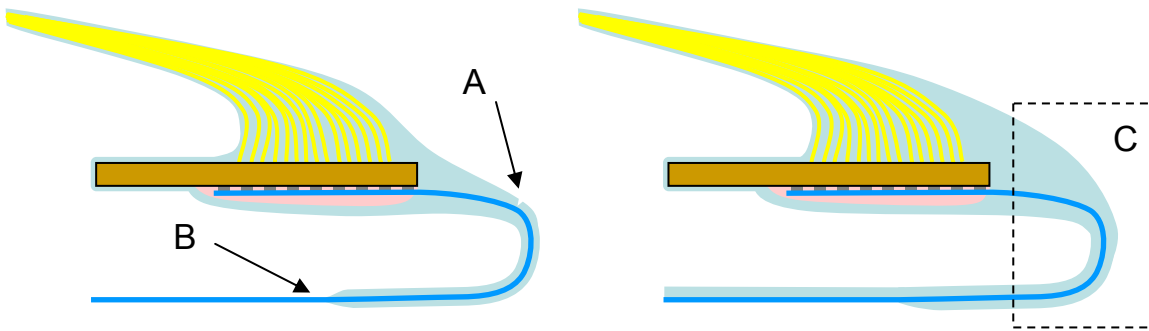


Figure 3. Intermediate designs. Areas of stress concentration (A and B) and improvements in the design (C).

Another problem observed with this implant design was that some rats lacked normal spinal reflexes for a few weeks after surgery. During explantation, it appeared that the thicker silicone made the implant stiffer in the region marked C in **Figure 3**, resulting in a spring effect that applied pressure on the spinal cord. **Figure 1** shows the current design, which involves a process that thins the protective silicone layer on the electrode array and uses a silicone overhang to protect the U-turn from any external pressure by surrounding soft tissue. Since the U-turn is much more flexible with this approach, it no longer applies undue pressure to the spinal cord. A finished device is shown in **Figure 4**.

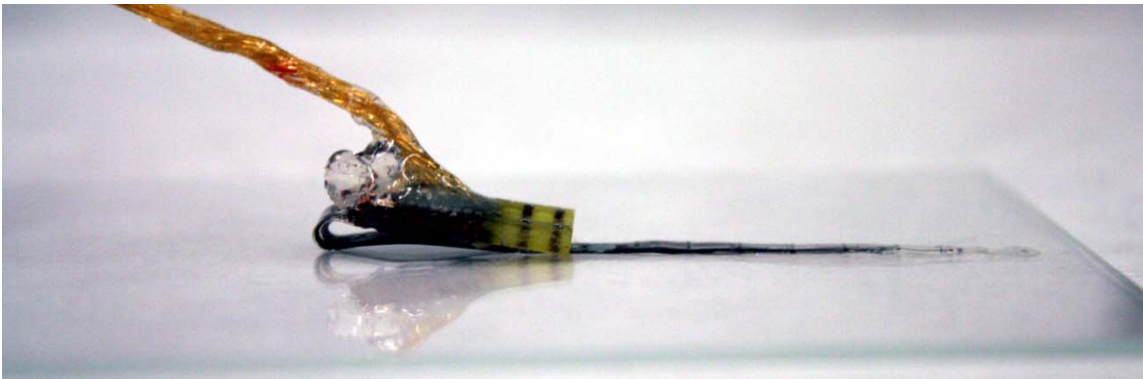


Figure 4. The current version of the chronic implant device.

We also found that multiple wires implanted under the skin with regular motion between the headplug and the entry point into the spinal column may cause skin damage and ensuing infectious complications. As we increased the number of signal wires in our complex array designs, the bundle of wires that must be routed under the skin between the array terminus in the lumbar region of the spinal cord and the headplug became substantially larger. The wire bundle was sufficiently large and stiff such that it often chafed against the skin of the animals when the animals were being trained or tested in the harness. To solve this problem we developed a new surgical technique for implanting the bundle of wires under the muscles to prevent direct contact between the wires and skin. Our recent experiments demonstrate that wires implanted in this manner remain stable, produce less damage to the skin, and reduce the risk of infection.

B. Microelectrode design

The implant structure is designed to minimize the mechanical stress placed on the microelectrode array during surgery implantation and normal motions of the rat. The most critical problem of the microelectrode

design, however, is that during the post-implantation period of approximately 12 different implantations we have observed progressive electrode connectivity failures over a period of 2-6 weeks. Based on these experiments, we have identified several specific factors underlying electrode failure, as well as solutions for each of these factors. We found that while some earlier designs of the arrays (**Figure 3**) yielded moderate success, there was evidence of poor electrode adhesion to the parylene substrate (**Figure 5**).

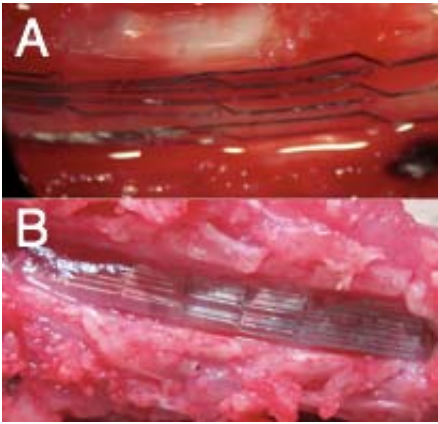


Figure 5. Delamination of electrodes. **A.** Example of detachments from the electrode pad base. **B.** Creasing of the parylene array substrate when implanted with silicone.

Initially the platinum on the electrodes was thickened using platinum electroplating to mitigate loss of the electrode during current injection, but it was found that this created a strong mechanical mismatch between the parylene substrate and the electrode surface (**Figure 6**). This had a tendency to concentrate stress at the edges of the electrodes when the rat was moving, therefore electroplating was no longer used.

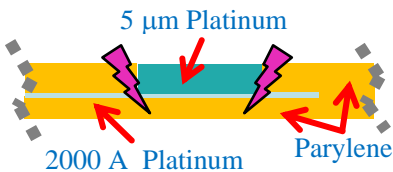


Figure 6. Mechanical mismatch causes stress at the electrode boundaries. Schematic illustrating electrode instability.

To solve this problem, several modifications have been suggested, developed, and/or tested:

1. **Argon-lon etch of parylene prior to platinization.** This additional process could provide additional cleaning of the parylene surface, and also provide additional free radicals at the surface, promoting better adhesion between the parylene and platinum layers. Surface treatment of the parylene beneath the platinum with argon plasma (as opposed to the usual oxygen plasma) was attempted but found to have no significant impact.
2. **Thinner platinum layer.** Reducing the electro-deposited layer will obviously lower the stress concentration effect. To compensate for changes in impedance, the platinum will be lightly doped to modify its electrical properties.
3. **An adhesion layer of 100 angstroms of titanium on the parylene prior to platinum deposition.** This additional process appears to be beneficial, as the most recent implants used arrays with this modification and have shown most electrodes to be functional well over one month after implantation, as opposed to the 2-3 weeks achieved with earlier designs.
4. **Parylene surface grid.** Parylene grid(2-□) on top of the electrodes was design to help mechanically limit initiation and propagation of electrode delamination. In implants containing electrodes with and without such a grid, delamination was found more frequently on electrodes without the grid (**Figure 7**). These

images show ripples in the platinum surface, but these are caused by exposure to hydrochloric acid used to dissolve tissues adhering to the implant after explantation.



Figure 7. After being implanted for 8 weeks, electrodes with a protective parylene grid (left) were in better condition than electrodes without the grid (right) where most of the delamination was observed.

Further tweaks to the design will continue, as the traces running between the baseplate and each electrode have shown to break on occasion, resulting in 2-7 electrodes per implant gradually losing functionality. Nonetheless, the current design has shown satisfactory reliability for the duration of the experiment (6-8 weeks).

Aim 2: Characterize the basic properties of the stimulating array developed in Aim 1, and establish effective stimulation protocols for enabling stepping in SCI rats using these high-density EAs.

By testing our chronically implanted design we found that most of the aspects of the system design worked well. We showed that the flexible high-density stimulating arrays provide better stimulation, as judged by locomotor performance and speed of locomotor recovery, than conventional wire epidural stimulating electrodes. **Figure 8** shows the results of stimulating the dorsal cord using various combinations of bipolar vs. monopolar stimulation configurations, as well as the effect of more ventral vs. more caudal stimulation foci. As seen in the step kinematics diagrams in both columns, stimulation through some combinations of electrodes can elicit active stepping. Stepping recovery was observed within 1.5 weeks of initial implantation of the arrays after the spinal cord injury, vs. 2.5 weeks after the injury with conventional wire stimulating electrodes.

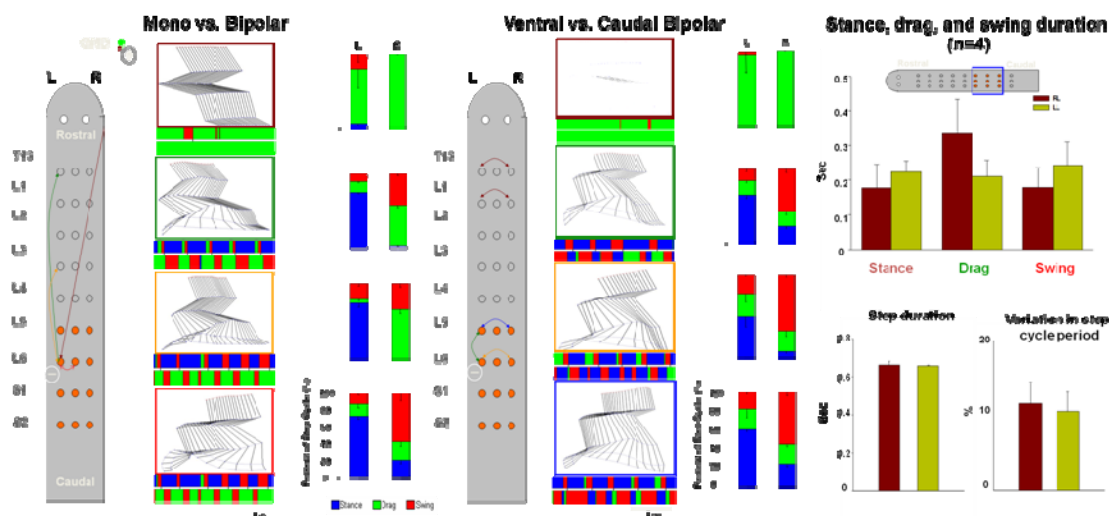


Figure 8: Effect of selective epidural stimulation with multi-electrode array system.

Aim 3: Test the hypothesis that high density ES, coupled with robotically-guided physical training will result in better stepping performance than ES alone or robotic training alone.

To address SA3 we have developed a spinal cord stimulation system integrated with multi-electrode array design that helps us to eliminate the problems related with chronic implantation of multiple wires design. We also have identified a new algorithm for spinal cord stimulation with array and machine learning paradigms that have the potential to more rapidly find the optimal array stimulation parameters.

A. Spinal cord stimulation system.

We have successfully developed a spinal cord stimulation system integrated with multi-electrode array design. This system includes having computerized control of the stimulation, and potentially we hope to have most of the electronics implanted and embedded in the spinal baseplate that will let us reduce the number of wires going to the headplug and possibly eliminate them altogether using a wireless design. **Figure 9** shows the layout and functioning prototype of a circuit that can route stimulation and recording signals from the implant to the appropriate data acquisition system. It is composed of 8:1 high voltage multiplexers (labeled M1-M9) that connect to the signals from the array as well as to the EMG signals, four shift registers at the top of the diagram used to configure the multiplexers with a serial data stream in under one microsecond, four low noise preamplifiers, and a few other power components (not shown). The circuit can be set up to stimulate or record between almost any two electrodes or amplify the signal from four electrodes or EMG wires simultaneously. The 27 electrodes, 2 ground wires, and 16 EMG wires (45 wires total) then are functionally reduced to one stimulation line and 4 amplifier signals, an enable signal, clock and data signals to configure the circuit, and power and ground lines (10 lines total). Because the circuit can be configured very rapidly, it can use time division multiplexing to record/stimulate more channels in a manner that is effectively simultaneous as far as the biology of the rat is concerned.

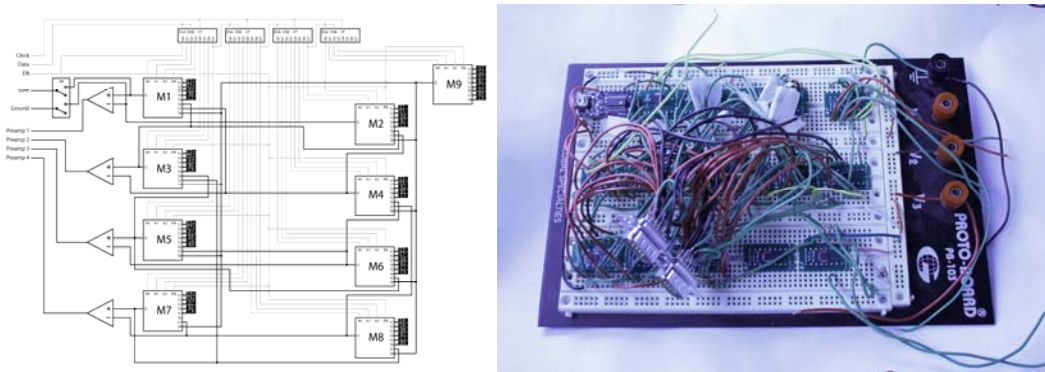


Figure 9. Multiplexer circuit and breadboarded prototype.

External multiplexer system was tested and successfully integrated in small implantable multiplexer/switching system. The multiplexer system multiplexes stimulating signals from the external electronics to different pairs of array electrodes. Using this device the number of array signal wires is reduced substantially, allowing for a smaller wire bundle to be routed to the head connector. This reduction allows for the use of our original head connector (8-12 pins) that is easier to implant and potentially allows for a wireless design. Implantable multiplexer circuit was designed and prototyped **Figure10**.

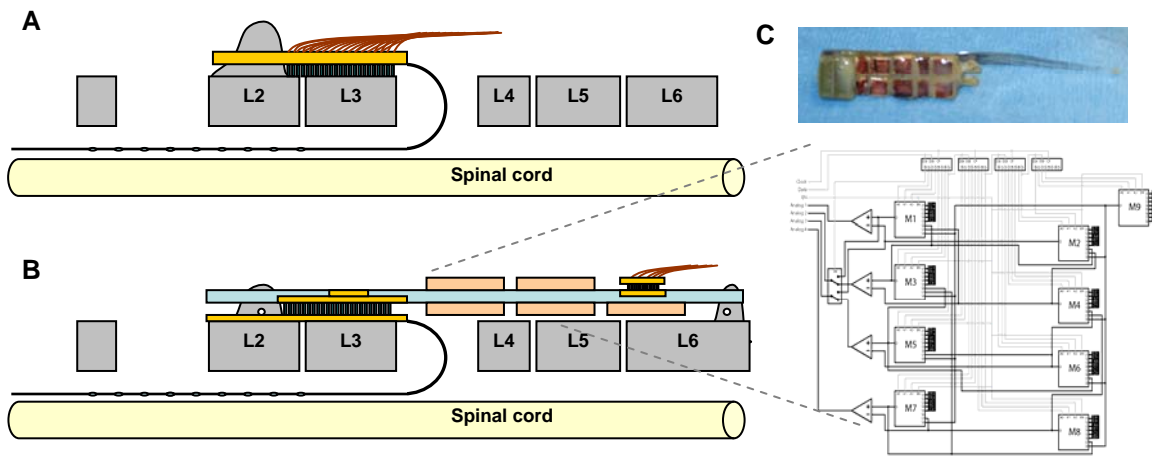


Figure 10: Multiplexer/switching system. A shows the geometry of the original array and wire bundle configuration. B shows the new array system configuration with embedded multiplexing chips (whose circuit diagram is shown at the right of B). C is a photograph of the assembled multiplexing system consisting of several multiplexing chips.

To control new system custom software was written (**Figure 11**). This software interfaces with a data acquisition unit that outputs the correct serial data stream to configure the circuit as well as control the stimulation. It was tested successfully when paired with the prototype multiplexer circuit.

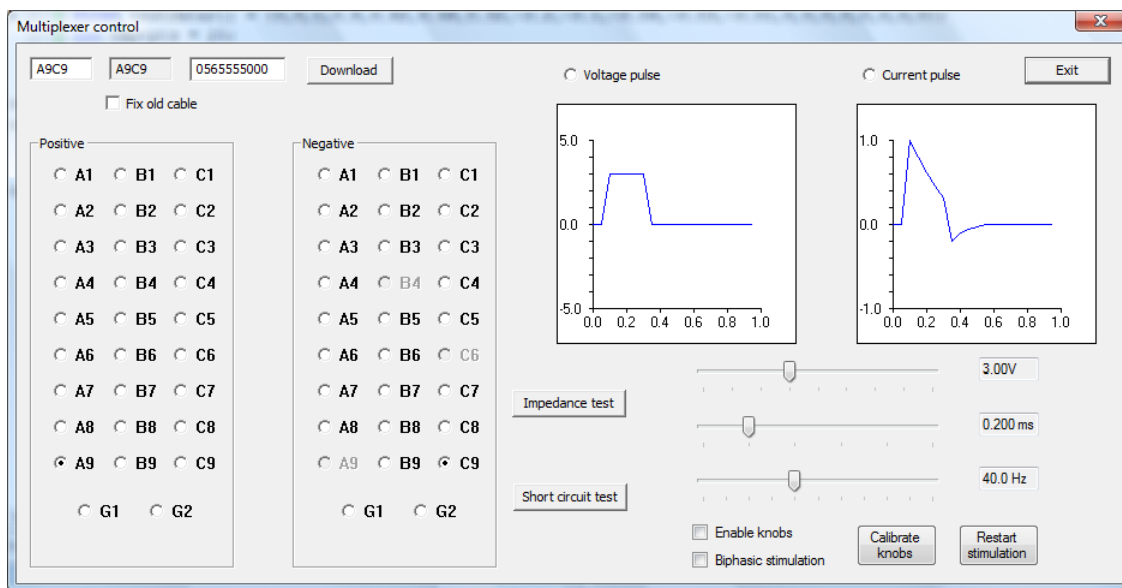


Figure 11. Multiplexer control software.

New surgical technique for implantation of the multiplexer/switching system was recently developed and tested. During our last chronic experiments we found that implantable multiplexer system appears to be functional after implantation and allows stimulate and records with similar parameters we observed in previous generation of array design.

B. Algorithm for spinal cord stimulation with array system.

Testing single motor evoked responses by stimulation with chronically implanted array design we have found that stimulation with the epidural electrode array could selectively activate spinal pathways responsible for monosynaptic and polysynaptic responses. Previously we reported that epidural stimulation with conventional wire electrodes induces three types of responses: an early response (direct stimulation of motor

pools or motor axons), a middle response (activation of monosynaptic pathways), and a late response (activation of polysynaptic circuitries). By testing selective electrode combinations on the array we found that bipolar stimulation of the caudal segments of the spinal cord can facilitate both early and middle responses at low thresholds, while monopolar stimulation between selected electrodes and the ground activates mainly the middle response at ~2x the intensity used for bipolar stimulation. We also observed that array technology allows selectively activate different spinal cord pathways to assess monosynaptic and polysynaptic circuitries. Thus, stimulation of the ipsilateral side of spinal cord induces both polysynaptic and monosynaptic responses. The same stimulation of the contralateral side of spinal cord induces only polysynaptic late responses. Altogether, these findings indicate the possibility for selective activation of different spinal pathways using this array technology. This provides novel perspectives for fine-tuning of the stimulation protocol and for the precise assessment of spinal circuits after a spinal cord injury.

Publications:

1. Gad P, Woodbridge J, Lavrov I, Gerasimenko Y, Zhong H, Roy RR, Sarrafzadeh M, and Edgerton VR. Electronic spinal bridge to facilitate stepping after a complete spinal cord lesion. *Journal of Neural Engineering*, 2010.
2. Gad P, Woodbridge J, Lavrov I, Gerasimenko Y, Zhong V, Roy RR, Sarrafzadeh M, Edgerton VR. Development of an electronic spinal bridge between the forelimbs and hindlimbs to facilitate quadrupedal stepping after a complete spinal cord transection. *Society for Neuroscience*, 2010.
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